Epithelium-Dependent Regulation of Smooth Muscle Contractility of the Airways

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The contribution of calmodulin and protein kinase C to the regulation of epithelial relaxing factor production by the tracheal epithelium and the role of adenylate and guanylate cyclase in the realization of the effect of this factor on airway smooth muscles are studied by the mechanographic method with cascade perfusion. Calmodulin and protein kinase C are shown to participate in the production of relaxing factor by epitheliocytes, guanylate cyclase being the principal target in exposure of smooth muscles to epithelial relaxing factor.

Key Words: *epithelium; trachea; smooth muscles; contraction*

Recent studies have confirmed the capacity of airway epithelium to modulate the contractile reactions of smooth muscles [4,8,10]. By analogy with the function of vascular epithelium, the mechanism of the modulating effect may be related to the production of epithelial relaxing factor (ERF). Available data permit a general description of the mechanism of epithelial regulation of the contractility of vascular smooth muscles [3], but this concept evidently cannot be extrapolated to the epithelial-smooth muscle relationships in the airways. Specifically, the mechanisms of calcium-dependent regulation of ERF production are unknown. The type and role of interactions between the adenylate and guanylate cyclase signal systems in the course of epithelium-induced bronchial dilatation also require elucidation.

In this research we studied the contribution of calmodulin (CM) and protein kinase C to the regulation of ERF production by tracheal epithelium and the role of adenylate and guanylate cyclase in the realization of the effect of ERF on the muscles of the airways.

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MATERIALS AND METHODS

Ring segments 3-4 mm wide and a 30-mm-long tubular preparation were cut from the trachea of outbred male white rats. The segment intended as the "acceptor" was mechanically deepithelialized as described previously [5]. The preparations were placed in a device for cascade perfusion [3]. Aerated Krebs' solution was delivered to the ERF "acceptor" at a rate of 0.5 ml/min through the lumen of the tracheal tubular preparation (ERF "donor"). The mechanical tension of the "acceptor" was measured using a 6MX2B mechanotron under conditions approaching isometric.

Calcium ionophore (A23187, 0.5 μM), added to Krebs' solution perfused through the "donor" lumen, initiated the production of ERF. The effects of ERF on the "acceptor" were assessed as the percent share of mechanical tension reduction in response to perfusion of hyperpotassium (30 mM) Krebs' solution. Krebs' solution of the following composition (mM) was used in the study: 120.4 NaCl, 5.9 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 15.5 $NaHCO_3$, and 11.5 glucose, pH 7.35 at 37°C. The test solutions were based on

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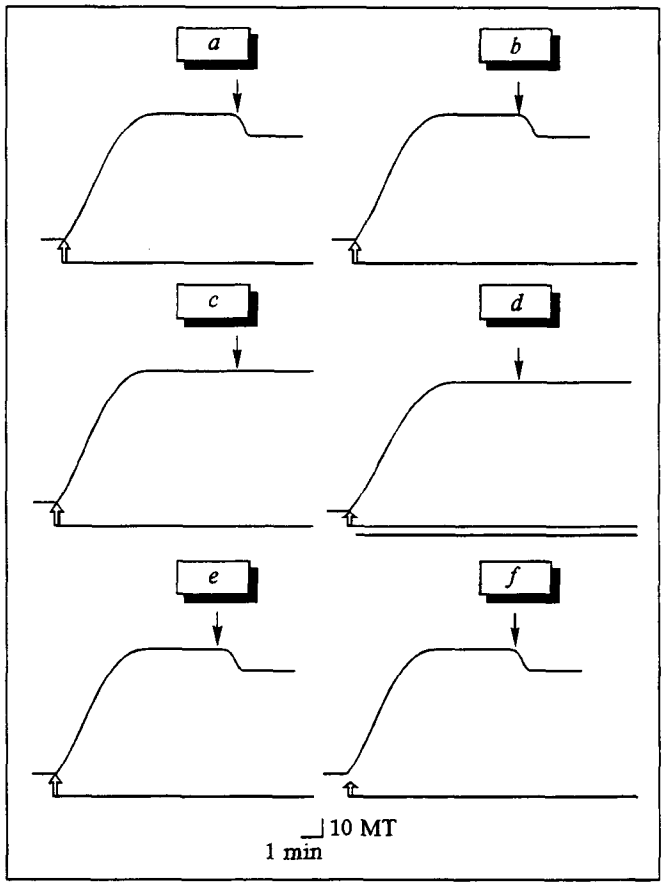

Fig. 1. Effect of Ca^{2+} ionophore A23187 (0.5 mM) (shown with an arrow) on mechanical tension of a tracheal segment after exposure of "donor" epithelium to test solutions (shown with a light arrow): methylene blue, 50 μM (*b*); trifluoroperazine, 10 μM (*c*); staurosporine, 0.2 μM (*d*); H−9, 0.5 μM (*e*); theophylline, 2.5 μM (*f*). (*a*: no pretreatment.)

Krebs' solution: 1 to 10 μM trifluoroperazine, 10 to 100 μM chloropromazine, 0.1 to 1 μM imipramine, 50 μM methylene blue, 0.5 μM H-9, 0.02 to 0.2 μM staurosporine, 0.5 to 2.5 μM theophylline, and 10 mM tetraethylammonium.

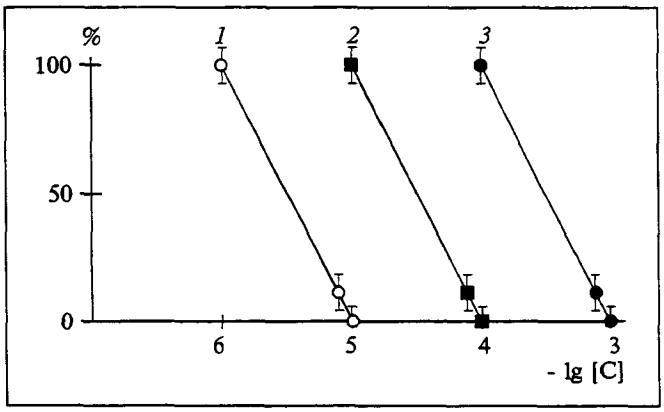

Fig. 2. Relationship between mechanical tension of rat tracheal segments and concentrations of trifluoroperazine (*1*), chloropromazine (*2*), and imipramine (*3*) in test solutions (pretreatment of donor epithelium).

RESULTS

Calcium ionophore did not affect the mechanical tension of epithelium-free segments. On the other hand, it caused relaxation of the acceptor preparation to 75% of the initial level during cascade perfusion (Fig. 1, *a*). This is in line with reports about Ca^{2+}-dependent regulation of ERF production by epithelial cells [13].

Known inhibitors of CM-activated reactions were used to investigate one of the mechanisms of Ca regulation of ERF production. A dose-dependent suppression of ERF production by CM antagonists was observed (Fig. 2), their activities ranking as follows (from highest to lowest): trifluoroperazine, chloropromazine, imipramine. The order of the series ranked by the EC_{50} value correlated with their affinity to CM. It can thus be stated with assurance that Ca^{2+}-CM-dependent processes contribute to the regulation of ERF production [1].

Another means by which the effects of a raised intracellular Ca^{2+} level are realized is protein kinase C activation [12]. Protein kinase C inhibitor in a concentration of 0.02 μM completely suppressed the relaxing effect of A23187 on the acceptor trachea (Fig. 1, *b*). The modulation of the activity of protein kinases unrelated to the rise of the intracellular concentration of Ca^{2+} did not affect the production of ERF. The lack of an effect of protein kinase inhibitor (H-9), guanylate cyclase (methylene blue), and cyclic nucleotide phosphodiesterase (theophylline) on the A23187-induced relaxing effect is further evidence in favor of this (Fig. 1).

For studies of the mechanism by which ERF acts upon smooth muscles, the acceptor trachea was pretreated with test agents. Figure 3, *b* shows that the guanylate cyclase inhibitor methylene blue completely suppressed the response to the test solution A23187 (0.5 μM). This is consistent with the hypothesis that guanylate cyclase is an ERF target [3,11]. H-9, an inhibitor of protein kinase A, had a similar effect on the acceptor (Fig. 3, *c*). This is indicative of cAMP participation in the epithelium-dependent relaxation.

Phosphodiesterase capable of hydrolyzing both cyclic nucleotides is the most probable site of interaction between the cAMP- and cGMP-dependent systems [5,7]. Indeed, theophylline, a phosphodiesterase inhibitor, (2.5 μM), suppressed the relaxing effect of A23187 (Fig. 3, *d*).

An increase of protein kinase A activity may occur in many intracellular processes. Specifically, potassium channels may be the actuating system of

smooth muscles [2]. Experiments with the potassium channel blocker tetraethylammonium (10 μM) demonstrated no effect of A23187 on the mechanical tension stimulated by this agent (Fig. 3, *e*).

These data suggest that the process of ERF production by epitheliocytes is Ca^{2+}-dependent and regulated by CM and protein kinase C, among other things. Guanylate cyclase is the principal target in exposure of smooth muscles. Many authorities believe there to be a cGMP-dependent protein kinase activating the system of Ca^{2+} removal from the sarcoplasm [5,6]. Our findings also attest to a cAMP-dependent relaxation of the tracheal smooth muscles.

It is notewothy that our results are in many respects similar to those obtained in studies of the effect of endothelium on vascular smooth muscle. This does not contradict the idea of common mechanisms being at work in the epithelial- and endothelial-smooth muscle relationships in the respiratory and circulatory systems.

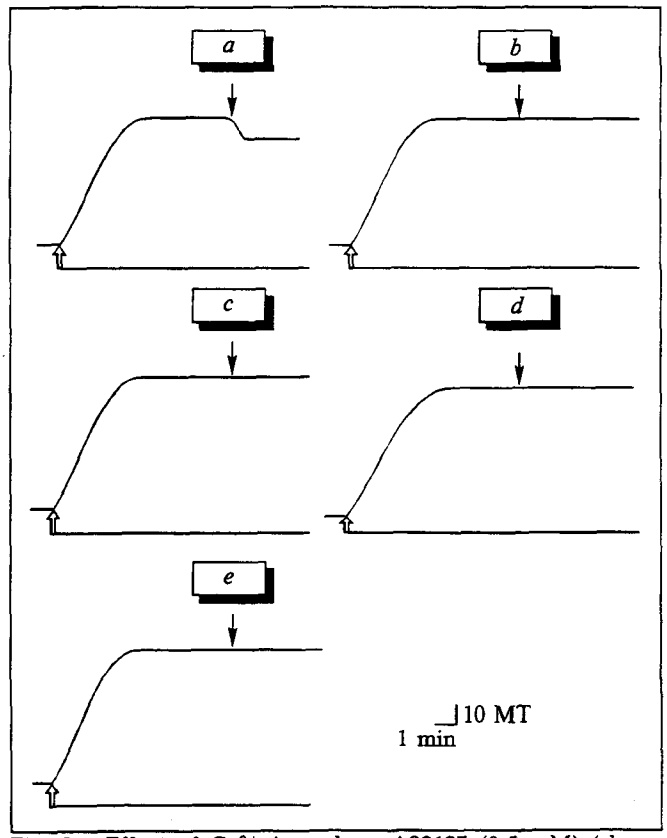

Fig. 3. Effect of Ca^{2+} ionophore A23187 (0.5 mM) (shown with an arrow) on mechanical tension of tracheal segment devoid of epithelium after pretreatment (light arrow) with test solutions methylene blue, 50 μM (*b*); H−9, 0.5 μM (*c*); theophylline, 2.5 μM (*d*); tetraethylammonium, 10 μM (*e*). (*a*: no pretreatment.)

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